physiology laboratory

Cell Culture Techniques:

A LABORATORY MANUAL

Compiled by,
Dinesh Thapa

Cell Culture Techniques

Introduction
Cell culture has become one of the major tools used in the life sciences today. This guide is designed to serve as a basic introduction to animal cell culture.

What is Cell and Tissue Culture?
Tissue Culture is the general term for the removal of cells, tissues, or organs from an animal or plant and their subsequent placement into an artificial environment conducive to growth. This environment usually consists of a suitable glass or plastic culture vessel containing a liquid or semisolid medium that supplies the nutrients essential for survival and growth. The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called **Organ Culture**. When the cells are removed from the organ fragments prior to, or during cultivation, thus disrupting their normal relationships with neighboring cells, it is called **Cell Culture**.

Cell culture consumable

Cell Culture Systems

Two basic culture systems are used for growing cells. These are based primarily upon the ability of the cells to either grow attached to a glass or treated plastic substrate (**Monolayer Culture Systems**) or floating free in the culture medium (**Suspension Culture Systems**).

Subculturing

When the cells have grown <80% confluency, they must be subcultured.

1. Briefly check the condition of cells by observing under microscope.
2. Harvest the cells as gently as possible (using enzymes for adherent type cells).
3. Once released, divide cell suspension and place into new culture vessels.

Cryopreservation of cells

(Cultures to be cryopreserved should be healthy, free from contamination, and should be maintained in log phase growth for several days before freezing.)

1. Trypsinize and centrifuge cells
2. Treat cells with suitable cryoprotective agents, such as DMSO or Glycerol (60% medium, 30% FBS, 5-10% DMSO).
3. Place the cells into cryopreservation ampules
4. Freeze the cells at a rate of 1℃/minute to a -70℃.
5. Transfer the ampules to a liquid nitrogen-filled storage vessel.

Cryogenic vials for freezing cells for long term storage

Storing cells in liquid nitrogen-filled storage vessel

Experiment: Resuscitation of Frozen Cell Lines

(Some cryoprotectants, such as DMSO are toxic above 4ºC therefore it is essential that cultures are thawed quickly and diluted in culture medium to minimize the toxic effects.)

1. Check technical data sheet
2. Prepare flasks by adding pre-warmed media
3. Collect cells (take out from liquid-nitrogen vessel)
4. Allow to thaw on waterbath (37℃) with gentle shaking by hands
5. Centrifuge ampule at low speed
6. Remove the medium
7. Re-suspend the cell pellet with fresh medium containg serum
8. Pipette cells into pre-warmed growth medium (dilute if required)
9. Incubate at 37℃ in 5% CO_2 containing incubator
10. Examine cells after 3-4 hrs (Change new fresh medium if required)
11. Examine cells after 24 hours

Experiment: Subculture of Adherent Cell Lines
(Adherent cell lines will grow in vitro until they have covered the surface area available or the medium is depleted of nutrients.)

1. View cultures using a microscope to assess the degree of confluency and confirm the absence of bacterial and fungal contaminants.

2. Remove spent medium.

3. Wash the cell monolayer twice with 10 ml of 1X HBSS

4. Pipette 200 µl trypsin/EDTA (working concentration 1X or 2X) onto the washed cell monolayer. Rotate flask to cover the monolayer with trypsin.

5. Return flask to the incubator and leave for 1-5 minutes.

6. Examine the cells using a microscope to ensure that all the cells are detached. The side of the flasks may be gently tapped to release any remaining attached cells.

7. Resuspend the cells in a small volume of fresh serum-containing medium to inactivate the trypsin. Perform a cell count (Cell Quantification).

8. Transfer the required number of cells to a new labeled flask containing pre-warmed medium

9. Incubate as appropriate for the cell line.

Experiment: Subculture of Suspension Cell Lines

1. View cultures using a microscope. Cells growing in exponential growth phase should be bright, round and refractile.

2. Do not centrifuge to subculture unless the pH of the medium is acidic (phenol red = yellow) which indicates the cells have overgrown and may not recover.

3. Take a small sample of the cells from the cell suspension (Cell Quantification). Calculate cells/ml and re-seed the desired number of cells into freshly prepared flasks without centrifugation just by diluting the cells.

4. Repeat this every 2-3 days.

Experiment: Subculture of HUVECs
(HUVECs and other primary endothelial cells are very loosly attached with the surface so suitable coating agents like gelatin, are required)

First coat the new flask with Gelatin: Add 2% gelatin solution and 20 ml sterile DPBS buffer. Incubate the flask at 37℃. After 30 minutes, decant the gelatin solution to waste bottle.

1. Observe the HUVECs under microscope and confirm the cells are in happy environment.

2. Discard the spent media to waste bottle.

3. Wash the cells once with 14 ml buffer saline.

4. Add 7 ml trypsin/EDTA, spread through the surface and gently shake the flask for detaching (Do not incubate the trypsinized cells in incubator).

5. After detaching the cells, add 14 ml neutralizing solution. Divide the cell suspension equal volume into 2 conical tubes (15 ml). Centrifuge for 1 min at 1000 rpm.

6. Discard the medium and add 5 ml fresh medium to re-suspend the cell pellet. Perform a cell count (Cell Quantification).

7. Transfer the required number of cells to a new labeled gelatin coated-flask containing pre-warmed medium.

8. Incubate as appropriate for the cell line.

Experiment: Cryopreservation of cells

1. Always choose healthy, contamination free and log phase growing cells for the preparation f cell stock.

2. Wash the cells, trypsinize and centrifuge.

3. Resuspend the cells in sterile 10% serum-containing culture medium.

4. Add 20% more serum to the above cell suspension.

5. Add 5-10% DMSO drop-wise with continuous shaking by hands. The DMSO must be of highest purity and molecular biology GRADE.(Thus final cell stock medium contains 50-60% medium, 30-35% Serum, 5-10% DMSO)

6. Place the 1 ml cell suspension to well-labeled cryo-vials (usually $2X10^5$ to $5X10^6$ cells/1ml ampule).

7. Then, transfer the vials into a box containing iso-propanol for control rate freezing (1℃/minute to a -70℃.)

8. Next day, transfer the stock vials to liquid nitrogen-fill storage vessels for prolonged storage.